TITLE:

Scaling of double and single stranded DNA : Exponents and Distributions(Knots and soft-matter physics: Topology of polymers and related topics in physics, mathematics and biology)

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CITATION:

Dietler, Giovanni. Scaling of double and single stranded DNA : Exponents and Distributions(Knots and soft-matter physics: Topology of polymers and related topics in physics, mathematics and biology). 物性研究 2009, 92(1): 75-79

ISSUE DATE:

2009-04-20

URL:

http://hdl.handle.net/2433/169115

RIGHT:

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Scaling of double and single stranded DNA

Exponents and Distributions.

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Long double stranded DNA (dsDNA) and single stranded DNA (ssDNA) molecules were studied by direct imaging with the Atomic Force Microscope (AFM) in order to determine their scaling properties, exponents, distributions and persistence length in the frame work of the theory of polymer physics and at the single molecule level. The effects of the topology on the statistical properties were investigated by considering linear, circular and knotted DNA molecules.

1 Introduction

Polymer physics [1] permits us to make predictions about the behavior of long thin molecules in respect to their size, shape and topology. This is well in tune with the needs of biology, which studies long DNA molecules and their behavior in living cells. The complexity of the biological systems (cell, nucleus, etc.) is way too difficult for a physical theory to take into account: details concerning sequence dependent properties, the presence of other molecules (from proteins downto simple chemicals) or active elements like enzymes (in our case topoisomerases) constitute important hurdles difficult to overcome. However, polymer physics has come up with a series of predictions enough general and not dependent upon details which can be compared to biological systems *in vitro*. On the other side, biological systems can be used as model systems to test the basic ingredients entering in the theoretical models and give us the unique opportunity to study, under controlled conditions of long monodisperse molecules, the assumptions made in any statistical mechanical calculation. Moreover, the computational methods are gaining importance due to the large systems they can handle and to the details that they can incorporate when doing numerical simulations, permitting to approach biological systems with more realistic models. But these simulations have to be tested against firmly demonstrated theoretical predictions and experimental confirmations. In this paper we present some results of the scaling behavior of long double stranded DNA (dsDNA) and single stranded DNA (ssDNA) molecules in 2 and 3 dimensions, their distributions and the effects of topology.

2 Materials and Methods

Linear DNA was prepared from a solution of λ-phage DNA, 48,502 base pairs (bp) long, cleaved by restriction enzymes to give a mixture of lengths from 1,503 bp up to the maximum 48,502 bp. Plasmids pUC19 (2686 bp), pBR322 (4361 bp), and pSH1 (5930 bp) were nicked to produce circular molecules without superhelicity. Plasmid pCU19 was treated with restriction enzyme RsaI to produce 3 different linear fragments, and using T4 DNA ligase mini-circles of different length were obtained. Knotted and unknotted DNA was isolated from P4 phage capsids

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according to the protocols given in [2, 3]. Circular ssDNA was purchased New England Biolabs. All DNAs were diluted to a final concentration of 1 $\mu g/ml$ in a buffer solution containing 1 $mM\ Tris-HCl$, pH 7.8 and stored at 4 ^oC. A 10 μl aliquot of dsDNA solution was deposited either onto freshly cleaved mica in the presence of 5 $mM\ MgCl_2$; weak adsorption [4] or mica modified with 3-aminopropyltriethoxysilane (APTES); strong adsorption [5], and incubated for 10 minutes at room temperature. Circular ssDNA was deposited on chemically modified graphite [6]. The sample was then rinsed with nanopure water and dried with air. Images were collected using an Atomic Force Microscope operated in tapping mode in air. Ultrasharp, non-contact silicon cantilevers with a nominal tip radius of <10 nm were driven at oscillation frequencies in the range of 150 to 300 kHz. During imaging, the surface was scanned at a rate of one line per second. Images were simply flattened using the AFM software, and no further image processing was done. The contour of the DNA molecules was extracted with *Ellipse* [7].

3 Results

We summarize here some results obtained on linear, circular and knotted DNA. In Fig. 1 are depicted AFM images of linear dsDNA, circular dsDNA, knotted dsDNA and circular ssDNA. The end-to-end distance as a function of the countour length is represented in Fig. 2 for linear and circular dsDNA. From these graphs, the critical exponent ν is extracted by fitting the data with the appropriate functions [5, 8, 9].

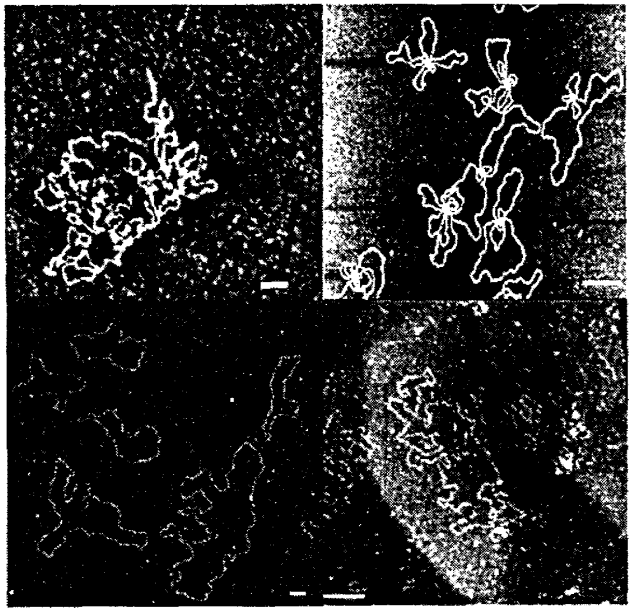

Fig. 1:AFM images of linear, knotted and circular dsDNA (top left, top right and bottom left, respectively). Circular ssDNA is depicted bottom right. Bar is always 100 nm.

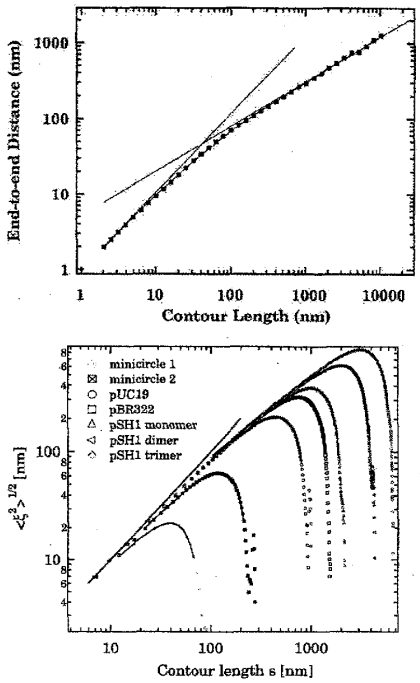

Fig. 2: End-to-end distance vs the contour length. Top linear dsDNA, bottom circular dsDNA.

Table 1 reports the values of the critical exponent ν found for the different samples we have studied. The mode of adsorption depends on the interaction between DNA and substrate on which DNA much adsorbed in order to be imaged by AFM. Strong adsorption causes a projection

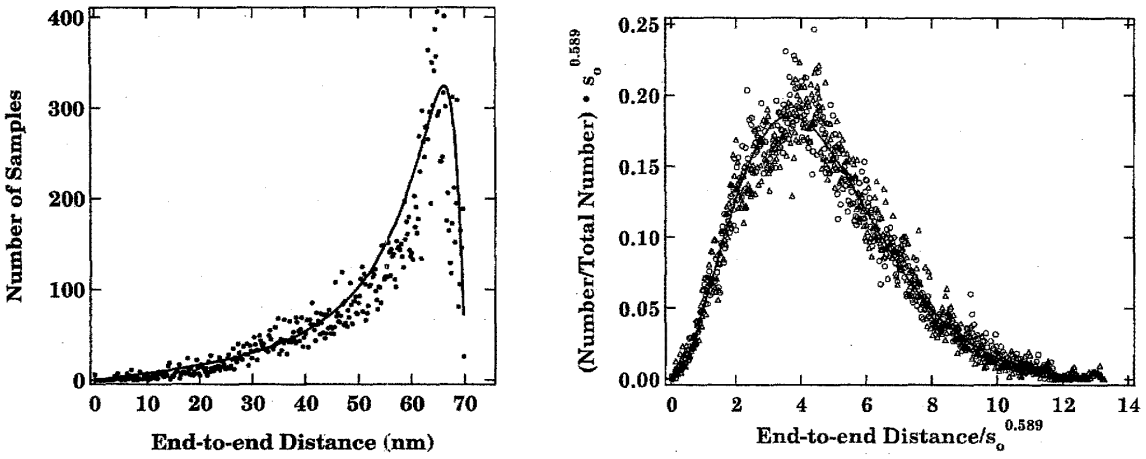

Figure 3: Distribution of the end-to-end distance for short length scales (left, $s = 75\ nm$), semi-flexible regime [10] and long length scales (right, $s = 548\ nm$ circles and $748\ nm$ triangles) SAW regime [11].

of the DNA on the substrate with a consequent 3 dimensional structure, while a weak adsorption permits to the DNA molecules a 2 dimensional relaxation.

Our results indicate that the topology of the DNA molecules does not influence the critical exponent ν: the values of ν are well within the theoretical predictions. It must mentioned, that the exponents are scale dependent: on short length scales (comparable to the persistence length ℓ_p) the DNA molecule is better described by a rigid or semi-flexible polymer models ($\nu = 1$), while on large length scales self-avoidance determines the behavior (in 2D $\nu = 3/4$, in 3D $\nu = 0.589$). The critical exponents reported in Table 1 are evidently measured in the limit of large lengths ($>> \ell_p$). The technique used to gather the data of Fig. 2 permits to get the contour of single DNA molecules: therefore one can also determine the distribution of the end-to-end distances for a given constant contour length. This permits to test the functions theoretically calculated. In Fig. 3 examples for the end-to-end distributions for linear dsDNA are give with their fitted theoretical distributions.

Table 1: Critical exponent ν for the strong adsorption (3D) and weak adsorption (2D) case.

Topology	Type		ν
Linear [5]	dsDNA	3D	$\nu = 0.588$
Circular [8]	dsDNA	2D	$\nu = 0.73$
Knotted [4]	dsDNA	3D	$\nu = 0.588$
Linear [12]	ssDNA	2D	$\nu = 0.73$

Similarly, distributions in the case of circular ssDNA can be determined: In Fig. 4 an example is give which was fitted with the function by [10],

$$f_s(\xi) = \frac{A\xi^2 e^{-3s/(4l_p(1-(\xi/s)^2))}}{(1-(\xi/s)^2)^{3/2}(2-(\xi/s)^2)^3}, \tag{1}$$

The data in Fig. 4 illustrate an other feature of the single molecule experiments together with Eq. 1. Namely, the persistence length ℓ_p can be extracted, not only from the bond correlation function, but also from the distributions. The data in Fig. 4 pertain to experiments with circular ssDNA: the persistence length extracted from these data is larger than what it was assumed up to now for ssDNA [12]. Persistence length of few nanometers were usually determined from pulling experiments or using fluorescence recovery experiments [13, 14, 15]. In our case a persistence length of 20 nm was obtained under conditions of low salt concentrations.

It is possible to determine the persistence length for higher salt concentration, and in the case of 10 mM $NaCl$ we obtained a persistence length $\ell_p = 10 - 12$ nm, higher than the 5 nm value found in literature. In fact, we have made the distribution using only parts of the ssDNA which do not show secondary structure or calculated the bond correlation function only for those parts. If we consider the whole molecule and calculate a global quantity like the radius of gyration (including parts of the ssDNA with secondary structure), we obtain persistence lengths similar to the literature values of $\ell_p = 5$ nm. We conclude that secondary structure determines the small persistence length at higher salt concentrations.

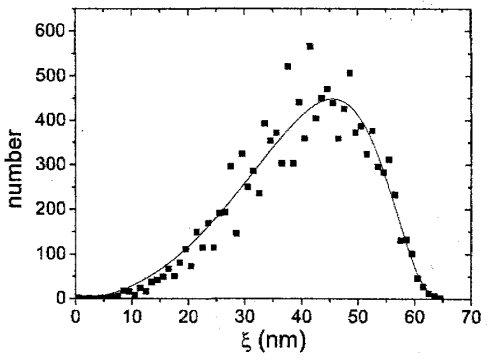

Figure 4: The distribution of end-to-end distances for a short contour length, $s = 66$ nm for 0.01 mM $NaCl$. The data are fitted with Eq. (1), resulting in a contour length of $s = 70\pm1$ nm, and a persistence length $l_p = 19.4\pm1.4$ nm.

Acknowledgement

The work presented here was performed with the support of the Swiss National Science Foundation and the Carlsberg Foundation. For the experiments presented here, I would like to acknowledge the contributions by Jozef Adamcik, Erika Ercolini, Mélanie Favre, Kristian Rechendorff, Francesco Valle, and Guillaume Witz. For discussions, advice and theoretical contributions to our work, I would like to thank Paolo De Los Rios. We benefitted of the constant advice and discussions with Bertrand Duplantier, Erwin Frey, Ralf Metzler, Roland Netz, and Andrzej Stasiak.

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